submitted that the Examiner consider the presently filed Rule 132 Declaration because the issues under 35 U.S.C. § 112, first paragraph, and the *In re Wands* factors are cited for the first time, and that the Rule 132 Declaration could not have been earlier presented. Alternatively, the Examiner is respectfully requested to enter this Reply After Final in that it places the application in better form for Appeal.

Applicants respectfully request the Examiner to reconsider the present application in view of the foregoing amendments to the claims.

Claims 35-61 are pending in the present application. In view of the following remarks, Applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims.

Withdrawn Claims

Claims 44-47, 58, 60 and 61 have been withdrawn from consideration as being directed to a non-elected invention. Applicants respectfully traverse this withdrawal.

Applicants respectfully request the Examiner to consider all instantly pending claims because the present withdrawal of claims is inappropriate. For example, Applicants respectfully submit that instantly pending claims 44-45 reflect subject matter in originally

filed claims 16-17 and 33-34, which were not previously restricted from the present application. Thus, Applicants respectfully request the Examiner to consider all instantly pending claims.

Issues under 35 U.S.C. § 112, First Paragraph

Claims 35-43, 48-57 and 59 stand rejected under 35 U.S.C. § 112, first paragraph, for asserted lack of enablement. Applicants respectfully traverse.

The Present Invention

The present invention involves quantitating the amount of peptide-IgG/IgE complexes and diagnosing aspergillosis based on the amount of peptide-IgG/IgE complexes. More specifically, the present invention is directed to a method for diagnosing aspergillosis in a patient, which comprises the steps of: incubating a body fluid sample from a patient with an ELISA plate having at least one peptide bound thereto; removing the body fluid sample from the ELISA plate; incubating the ELISA plate with anti-human IgG/IgE to form peptide-IgG/IgE complexes; removing IgG/IgE not bound in a complex; quantitating an amount of peptide-IgG/IgE complexes; and diagnosing aspergillosis based on the amount of peptide-IgG/IgE complexes. The at least one peptide is a peptide

comprising an amino acid sequence comprising one of SEQ ID NOS: 1-6.

The Present Specification Enables One Skilled in the Art in Making and Using the Present Invention

Generally, the Office Action refers to the *Wands* factors, and states that one skilled in the art can diagnose aspergillosis only with undue experimentation. For example, reference is made to how "no comparable methods for aspergillosis confirmation or diagnosis have been established" and "there is no way a priori of predicting actual diagnostic levels" (see Office Action at page 6, lines 2-4 and 10-11).

However, Applicants respectfully submit that no reference to outside sources (*i.e.*, comparable methods) is needed for diagnosing aspergillosis because the present specification already provides the information that one skilled in the art would need to make such a diagnosis (*i.e.*, sufficient information to make and use the present invention). Applicants herein enclose a Declaration pursuant to 37 C.F.R. § 1.132 by co-inventor, Puranam Sarma.

The Rule 132 Declaration first provides information on the state of the art (starting at page 4). Then, the presently submitted Declaration explains how the present specification

enables one skilled in the art in making and using the present invention (starting at the bottom of page 6).

1. *"Making" the Present Invention*

The starting material is readily accessible, wherein one skilled in the art can make the present invention. Reference is also made to parts of the present specification, wherein one skilled in the art can use the epitopes for diagnostic assays (*i.e.*, Examples 2 and 3; see pages 16-17 of the specification) in detecting *Aspergillus fumigatus* in patients via comparison tests. The data in Tables 2 and 3 in the present specification demonstrates diagnostic ELISA for aspergillosis in a patient, and the significant increase in the amount of immunoglobulin-peptide complexes (or binding) that form is an indicator for aspergillosis in a patient. The present specification and Rule 132 Declaration further teaches that the color of the end-product and that of the aspergillosis patient (see discussions of procedure that includes ELISA at page 8 of the Declaration) will vary. Thus, there is sufficient description in the present specification of making the present invention.

2. *"Using" the Present Invention*

There is also sufficient description in the present specification of using the present invention.

The formation of the colored product leads to the diagnosis that the person is suffering from aspergillosis. A peptide will react with the sera of an aspergillosis patient, which forms a colored product due to the increase in IgG or IgE levels when compared to normal sera. The comparison is based on the immunoglobulin-peptide complex, and is an indicator of aspergillosis in a patient. Thus, one skilled in the art would understand that the levels of Ig binding of the control patient are compared to that of the aspergillosis patient.

As one example, Table 2 shows significant increases (ten-fold; fifteen-fold) in IgG levels for those with aspergillosis. One skilled in the art would understand such comparisons are sufficient indicators of aspergillosis.

Thus, the present specification already provides the information that one skilled in the art would need in diagnosing aspergillosis in a patient (*i.e.*, sufficient information to making and using the present invention).

Conclusion

Thus, Applicants respectfully submit that one skilled in the art, upon reading the specification, can make and use the present invention without undue experimentation. Applicants respectfully request the Examiner to consider the experimental data in the present specification, as well as the instantly submitted Rule 132 Declaration as evidence of such enablement. Therefore, Applicants respectfully request the Examiner to reconsider, and to withdraw this rejection and allow the currently pending claims.

A full and complete response has been made to all issues as cited in the Office Action. Applicants have taken substantial steps in efforts to advance prosecution of the present application. Thus, Applicants respectfully request that the Examiner pass the application to issue.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Eugene T. Perez (Reg. No. 48,501) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By #32,888
for Mark J. Nuell, #36,623

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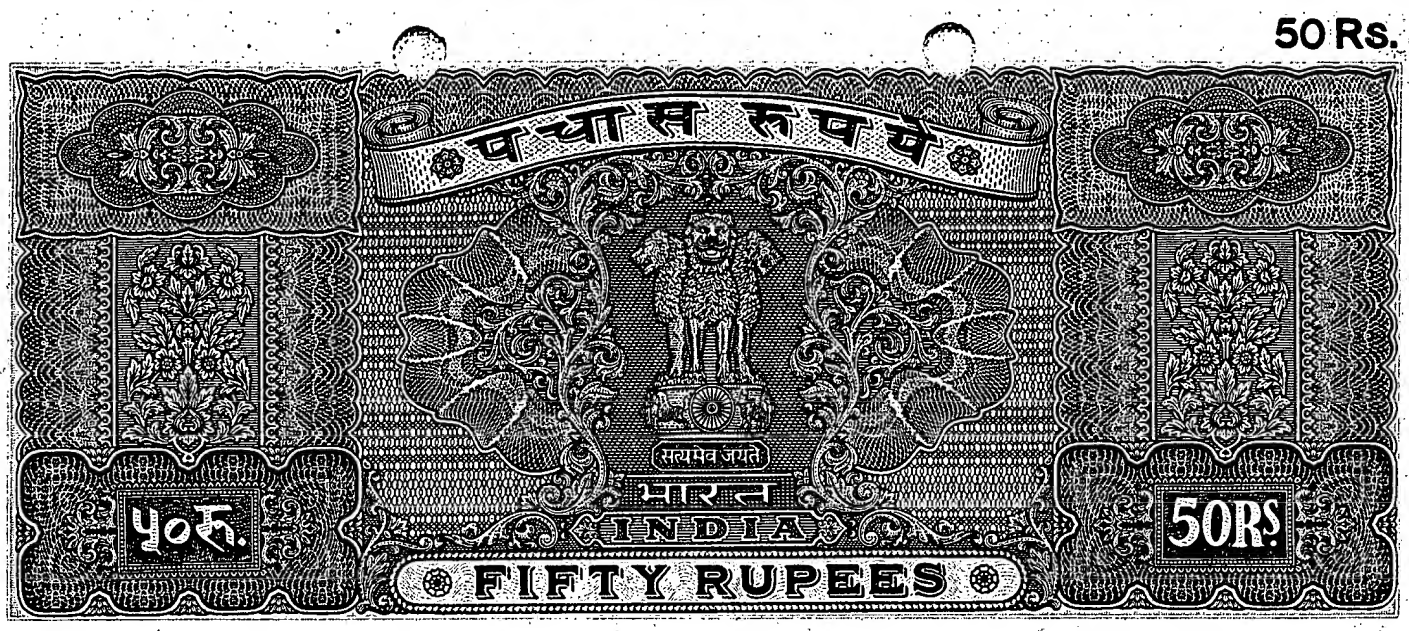
DRN/ETP/enm
2761-0147P

Attachment: Declaration under 37 C.F.R. § 1.132

(Rev. 04/30/03)



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IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant :	Puranam U. Sarma et al	Conf.:	5257
Appl. No. :	09/871,961	Group :	1631
Filed :	June 4, 2001	Examiner :	L.CLOW

For: NOVEL POLYPEPTIDES USEFUL FOR DIAGNOSIS OF ASPERGILLUS FUMIGATUS AND A PROCESS OF PREPARING THE SAME

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, DC 20231

I, Puranam U Sarma, residing at A-126, Inderpuri, New Delhi-110012, a citizen of India, do declare as follows:

1. I am a scientist at Centre for Biochemical Technology, Mall Road, Delhi, India. I graduated in the year 1964 from Osmania University, India. I completed my Master's Degree in Biochemistry from Osmania University at Hyderabad, India, in the year 1966. Subsequently, I was graduated with a doctoral degree in Biochemistry from Osmania University in the year 1974.
2. After completing my doctoral degree, I took up my first assignment as a Scientist with Centre for Biochemical Technology (from Nov 1980 to till date). Subsequently, I took up the post of Scientist with

Sarma

Sr. No. 9311 Date 6/5/03

Sold to Institute of Genomics & Integrative Biology

R/o ..... formerly Centre of Biochemical Technology

In Favour of ..... Council of Scientific & Industrial Research

Purpose Mall Road Delhi University Campus Delhi.

Through Declaration Ramp

BHOJRAJ LIC No 369
Bihari Colony, Delhi-32

6/5/03

I served this company for 22 and a half years. I joined the CBT in the year 1980. Currently, I am working as a Senior Scientist with CBT.

Thus, I have been working in the field of immunology for the last $22\frac{1}{2}$ years.

3. I am listed as one of the inventors of the subject of the above-identified application, and I have read and understand the application. I am also aware and familiar with all the office actions, objections of the Examiner, and the references cited by the Examiner. Therefore, I am completely and fully aware of all the facts relating to the present patent application.

4. One of the projects undertaken by CBT is "*Novel polypeptide useful for diagnosis of aspergillus fumigatus and a process of preparing the same*". This project was undertaken in the year 1994. The scientists involved in the study were Puranam U. Sarma (myself), Taruna Madan, Priyanka Priyadarsiny, Seturan B. Katti, Wahajul Haq. I was one of the main scientists in this study, and I am completely and fully aware of all the facts relating to this project.

5. I now discuss the present invention and the state of the art.

Background and The Present Invention

Aspergillus fumigatus causes a wide spectrum of disorders such as allergic bronchopulmonary aspergillosis. Allergens and antigens of *Aspergillus fumigatus* have been identified by several workers, such as Teshima *et al.* (1993), Kumar *et al.* (1993), Moser *et al.* (1992), Arruda *et al.* (1992), and Banerjee *et al.* (1996). However, none of these allergens and antigens has been introduced as a diagnostic product in the market. Therefore, prior to 2001, there was a dearth of diagnostic kits for detection of aspergillosis.

The present invention addresses this scarcity problem. The present invention provides novel peptides of *Aspergillus fumigatus*, and also provides an immunodiagnostic ELISA kit based on the antigenic peptides.

In particular, the present invention provides epitopic peptides of 18 KD allergen of *Aspergillus fumigatus* strain No. 285 isolated from the sputum of a patient having allergic bronchopulmonary aspergillosis. The strain has been deposited at American Type Culture Collection (ATCC) with Accession No. 42202. Five epitopes were identified on the 18 KD allergens in the immunodominant region from amino acid positions (aa) 6-22, comprising aa 10-20, aa 6-20, aa 14-20, aa 10-22, aa 6-13. The epitopic sequences were synthesized by solid phase method. The present inventors were able to bind *A. fumigatus* specific antibodies in the sera of patients and hence, are useful in enzyme-linked immunosorbent assay (ELISA) for the diagnosis of aspergillosis. These peptides exhibited

immunogenic properties and hence have potential application in immunotherapy.

State of art

At the time this project was undertaken, i.e., in the year 1994, a person skilled in the art, i.e., a person working in the field of immunology and immunotherapy, would be a person holding at least a Master's degree in Biochemistry, Biotechnology, and/or Immunology. The person skilled in the art would be familiar with methods relating to the isolation and purification of peptides from a given sample.

When given samples, as such sputum, the person skilled in the art would be in a position to purify and isolate these peptides, especially as directed by the specification of the present application.

Some of these steps involve in the isolation of the peptides are as under:

IDENTIFICATION OF EPITOPES, SYNTHESIS OF PEPTIDES (BASED ON EPITOPIC AMINO ACID SEQUENCE), PURIFICATION OF PEPTIDES (HPLC) CHARACTERISATION OF PEPTIDES BY FABMS.

ELISA as a general concept that was known to a person skilled in the art at the time this project was undertaken, and definitely prior to the date of filing of the present application (i.e., November 3, 1998, the date of the parent application No. 09/2184,938). ELISA is an assay in which a series of specific antibody-antigen and antibody-antibody interactions are used to bind enzyme molecules (*e.g.*, horseradish peroxidase) to the bottom of a 96-well plate in such a way that the amount of enzyme is directly proportional to the amount of antigen in the system. The amount of enzyme activity is then measured using a color-producing substrate (*e.g.*, ABTs). From this, the amount of antigen can be calculated. Therefore, with ELISA, an enzyme conjugated to an antibody reacts with a colorless substrate to generate a colored reaction product.

A number of variations in the ELISA technique have been developed to determine antigen/antibody. The assay used in the present invention is the indirect ELISA method. An indirect ELISA is used to detect or quantitate antibodies (or conjugate antibodies). Serum or other samples containing primary antibodies is added to an antigen-coated microtiter well, and allowed to react with the bound antigen. Then, free antibody is washed away. The presence of antibody bound to antigen is detected by adding an enzyme conjugated anti-isotype antibody that binds to the primary antibody. Free secondary antibody is washed away and a substrate for the enzyme is added. The addition of substrate generates

a colored reaction product that is measured by specialized spectrophotometric plate readers, which can measure the absorbance of the well plate in less than a minute. The amount of binding is directly proportional to the color generated in the end-product.

The ELISA and indirect ELISA methods are generally explained by Janis Kuby in the book titled "IMMUNOLOGY" (Second Edition, W.H. Freeman and Company (1994)).

Before the date of filing of the present application, the general nature of *Aspergillus fumigatus* was known. As mentioned earlier, a few peptides from this fungus have been isolated by just a few workers. However, no reliable diagnostic assay and kits were available in the market for determination and diagnosis of the aspergillosis. It is the invention of the present application that provides such an assay for the first time.

Thus, the level of skill required by a person regarding the present invention is general, and such a person is expected to be aware of and familiar with the methods for purification of peptides and ELISA as a general concept.

Disclosure in the specification

As a person skilled in the art, it is my view that the specification as filed generally teaches how the invention is to be performed. Also, the state of the art is such that no adequate prior

methods existed that would give indications that a patient has aspergillosis.

a. *"Making" the Present Invention*

First, the present specification provides the starting material of the strain from which the peptides are to be isolated (the *Aspergillus fumigatus* strain No. 285). As mentioned, the starting material of this strain has been deposited at the ATCC (Accession No. 42202). The epitopic sequences in the immunodominant region of the 18 KD allergens are to be isolated by a method known in the art, including the solid phase method. Also, the present specification guides one skilled in the art to the structure and nature of the peptides. The sequence of the peptides has been given in the description; the sequence listing of the peptides has also been provided. The epitopes and their sequences have also been given in the present specification. Example 1 describes how the allergen is to be isolated for further tests. Thus, the starting material of the present invention has been adequately described.

Second, the present specification sufficiently guides one skilled in the art to making and using the present invention. Use of the epitopes in diagnostic assay is provided in Examples 2 and 3, wherein these Examples describe the use of the peptides in diagnostic assay for detection of *Aspergillus fumigatus* in patients by comparing with the

normal/control. Tables 2 and 3 set out the results of the assay of Examples 2 and 3.

Example 2 demonstrates that the synthetic peptides react with sera of aspergillosis patients. In Example 2, sera of a healthy person as well as a patient having sera of aspergillosis are tested. The method here involved, first, the ELISA plates being coated with the peptides obtained from the *Aspergillus fumigatus* strain No. 285 (the one deposited at ATCC). Second, the un-reacted sites are blocked with bound serum albumin. Thereafter, the sera of patient/control is added to the well and incubated. The plates are washed and anti-human IgG HRP conjugate is added. Subsequently, *Aspergillus fumigatus* substrate is added. With the addition of this substrate, a colored product is formed. The color is red, and spectrophotometric plate readers are used. The color is measured in terms of absorbance values (the amount of binding is directly proportional to the color generated in the end-product). The present specification teaches that the color of the end-product and that of the aspergillosis patient will vary.

b. "Using" the Present Invention

Thus, as can be seen from the disclosure in the present specification, the peptides reacts with the sera of aspergillosis patient, and this reaction or binding finally forms a colored product.

The formation of the colored product leads to a diagnosis that the person is suffering from aspergillosis.

Although there is no specific level of ELISA absorbance that can be considered as an indicator of aspergillosis in a tested patient, one skilled in the art can determine as to whether or not a patient has aspergillosis based on the present invention. The determination of aspergillosis is determined by the increase in the IgG or IgE level when compared to normal sera. The comparison is based on the immunoglobin – peptide complex and this serves as an indicator as aspergillosis in a patient.

In other words, the present invention guides one skilled in the art that the levels for IgG binding of the control patient must be compared with that of the aspergillosis patient. The IgG binding of the control patient may also be compared with IgE binding of the control patient with the IgE binding of the aspergillosis patient.

For instance, in referring to Table 2, it is apparent to one skilled in the art that the patient's sera has a significant increase in IgG and IgE levels when compared to the normal sera. As one example, by using peptide 2, the IgG level in a normal patient is 0.09, whereas the patient with aspergillosis has an IgG level of 0.852. This increase is nearly ten-fold, and is clearly an indication that the patient with the ten-fold increase has aspergillosis. Similarly, in case of peptide 3, the IgG level of a patient is only 0.011 whereas that of patient is

0.674; this increase is more than six times. The same is the case with the peptide Nos. 4, 5 and 6. Flow/valuation in Table 2 shows a four to twenty times increase in IgG and 10 to 1000 times increase in IgE. Therefore, the significant increase in the amount of immunoglobin - peptide complex that are formed serves as an indicator for aspergillosis in a patient.

With respect to Table 3 of the present specification, the level of antibodies in immunized mice is far greater than the control mice when the absorbance is read at 490 nm. The level of increase is about 11 times. The same kind of increase in the level of antibodies is seen with peptides 3 to 6 as well.

In view of the above discussion, as a person skilled in the art, I would believe that the specification of the present invention clearly sets out the method for diagnosis of aspergillosis using the peptides of the invention. It is very clear from Tables 2 and 3 in the specification that the levels of IgG and IgE serve as an indicator of aspergillosis. One skilled in the art would be aware how to interpret the readings of ELISA, and would there would be no undue burden in performing the present invention as described by the specification.

There is no undue experimentation involved with the present invention, as once the assay is performed as taught by Examples 2 and 3, the results can be easily read by a spectrophotometric plate reader. No further assays are required to determine whether or not a person is suffering from aspergillosis. The claims of the present application even require a reading of the absorbance values. Therefore, the present specification sufficient guides one skilled in the art to make and use the present invention without experimentation that would be considered as undue.

6. I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 7-5-2003 By P. Usha Sarma
Puranam U. Sarma